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AWARD NUMBER: W81XWH-14-1-0401

TITLE: Topical Modulation of the Burn Wound Inflammatory Response to Improve Short and Long Term Outcomes

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

We propose to investigate the relationship between p38MAPK signaling, wound inflammatory response, wound healing and long-term scar formation using a burn model in the female red Duroc pig. We hypothesize that topical p38MAPK inhibition will attenuate the depth of the burn by preventing hair-follicle cell apoptosis, attenuate the inflammatory phase of wound healing, and decrease the granulation layer thickness. We propose this modification in the early inflammatory response will also reduce thickness and contraction of scars formed after deep partial thickness burn injury. The knowledge gained from our proposed research will be critical to implement a potential paradigm shift in the clinical treatment of challenging dermal injuries.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:	17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
			USAMRMC

a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
Unclassified	Unclassified	Unclassified	Unclassified		

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Approximately 500,000 Americans suffer burn injuries with an estimated 3,500 deaths annually. Widespread makeshift bombs contribute to burns and large wounds being one of the significant causes of warfighter causalities. The magnitude and impact of burns can be devastating as large numbers causalities occur simultaneously. Secondary organ damage and failure frequently occurs after injury. Moreover, wound complications such as hypertrophic scars may cause significant morbidity, disabling loss of function, extended difficult recovery times, dramatically affecting the patient's quality of life physically. We are investigating a topical therapy that is easy to apply and can be used by a wider range of health care providers in a mass-casualty incident.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Wounds, Burn, topical, wound healing, inflammatory signaling, Mitogen activated protein kinase, hypertrophic scar, p38, combat casualty, treatment, organ failure, systemic inflammatory response syndrome, thermal injury, wound model, intervention

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

What were the major goals of the project?

1. Establish the female red Duroc pig model burn model as the appropriate wound healing model that resembles human response. At the end of the project, we will have a well-defined animal model for human wound healing. This animal model may provide a tool that other investigators can use to screen for compounds that may modify wound healing and reduce scar formation. The ability to have a standard animal model for wound healing may bring an exciting new era in the investigation and elucidating the molecular mechanisms of hypertrophic scar pathophysiology and developing therapeutic agents.

2. Define the inflammatory signaling post burn injury and elucidate the relationship between early inflammatory signaling, wound healing, and scar formation.

3. Define the role of p38MAPK in wound healing and scar formation.

4. Identify the wound healing response to topical p38MAPK inhibition. Demonstrate early wound healing and reduced scar formation with topical p38MAPK inhibition. Define the long-term wound outcome of topical p38MAPK inhibition post-burn injury.

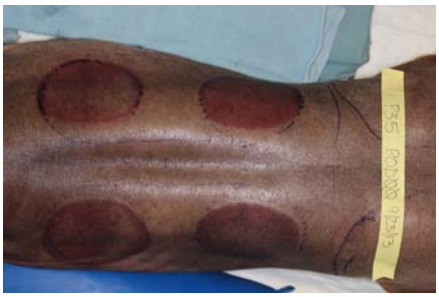
5. Identify the optimal timing and duration of treatment for p38MAPK therapy.

6. By the end of the project, have a well-defined protocol and experimental plan to initiate human subject research to study topical p38MAPK inhibition as a therapy to decrease end-organ dysfunction, improve wound healing, and reduce scar formation in patients with burn injuries.

What was accomplished under these goals?

Please look at the table 1. This is a list of porcine experiments that our group has conducted. The experiments started before the W81XWH-14-1-0401 grant was initiated (mark on the table). The first goal of the grant was to establish a burn wound healing model. Our group explored the female red Duroc porcine dermatome model and obtained serial biopsies of deep and shallow wounds at 1, 2, 3, 12 and 20 weeks, demonstrating that the female red Duroc porcine model significantly correlates to human hypertrophic scarring (period before the award of the current grant).

Table 1 Study ID	Wound	Dates	Treatment	Pigs #	Duration of study
Pg001	Dermatome	Feb-10	Topical p38MAPK inhibitor versus control	2	20 weeks
Pg002	Dermatome	Sep-11	Topical p38MAPK inhibitor versus control	2	20 weeks
Pg003	Dermatome	Sept 2012-Oct 2012	PGE2 agonist topical versus control	6	3 weeks
Pg004	Scald Bottle Burn	Sept 2013- Oct 2013	Topical p38MAPK inhibitor versus control	6	2 weeks
Pg005	Scald Bottle Burn	May 2014	Topical p38MAPK inhibitor versus control	6	2 weeks
DoD Grant Funded following:					
Pg006	Scald Bottle Burn	Dec 2014	Topical p38MAPK inhibitor versus control	6	3 days
Pg007	Scald Bottle Burn	May 2015	Topical p38MAPK inhibitor versus control	6	3 days
Pg008	Scald Bottle Burn	Sept-Oct 2015	Topical p38MAPK inhibitor versus control	8	2 weeks



We started our experiments using burn wound model with Pg004. In this model we use a 'hot water bottle' thermal injury device. Briefly, we use a 500 ml Pyrex laboratory Schott Duran bottle with the bottom glass removed, edges smoothed, bottom replaced with cling wrap, and secured with heat resistant tape. The bottles will be filled with 300 ml of water and then heated to the desired temperature of 92°C. We have improved our technique significantly over the period of the current grant. The initial wounds were not uniform. We have now resolved the technical issues and our Pg007 and Pg008 burn wounds are uniform. We change the depth of the burn by changing the length of contact 10, 15, and 20 seconds. We have identified that all these contact times are in the range of partial thickness injury. The 20 seconds is mostly very deep partial thickness burns with central area of full-thickness injury. We have analyzed these wounds using several different methods:

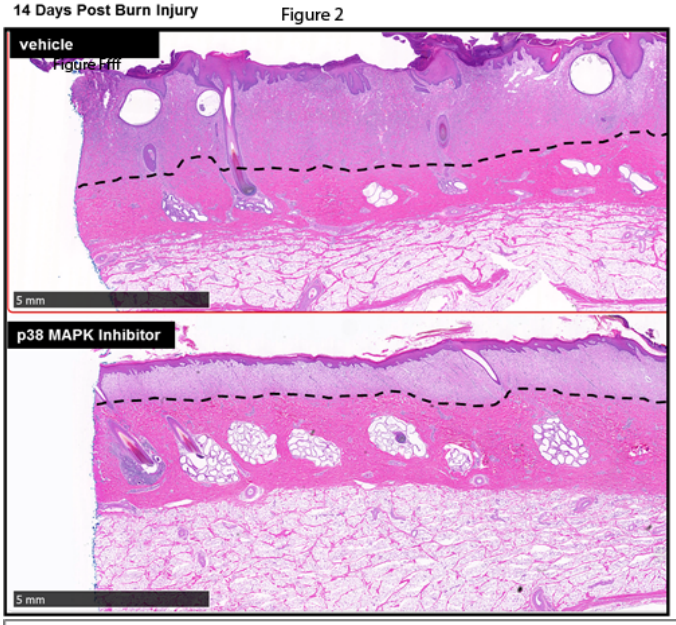
- Wound character: time to wound closure, color, wound infection
- Histopathology: H&E, TUNNEL assay,
- Inflammatory and wound healing gene expression

The burn wound healing is different than the dermatome wound healing, but they do share common characteristics. In both groups there is a portion of collagen arrangement that remains intact. In the dermatome model the line between "normal" collagen and "abnormal "collagen" is sharp and clear. In the burn model there is a large transition zone between the intact dermal architecture and damaged skin. This reflects the ongoing inflammation and apoptosis seen in burn injury. Figure 2 demonstrates two areas of deep

partial thickness burn with the dotted line demonstrating the intact architecture. When we examined the gene expression differences in various depth of injury, an interesting pattern was observed. The deeper burns were associated with increasing number of over-expression of the regulatory genes (figure 3). This internal consistancy in the model is very important. Table 2 demonstrates the various genes that we explored. The table compares 20 second burn to the uninjured skin. The differences between the matrix metallopeptidase and transforming growth factors are of particular importance. These genes have been suspected of being some of the pathways that may induce hypertrophic scarring. Considering our success in recent experiments with data supporting the model, we can state that we achived our first goal (Establish the female red Duroc pig model burn model as the appropriate wound healing model).

14 Days Post Burn Injury

Figure 2



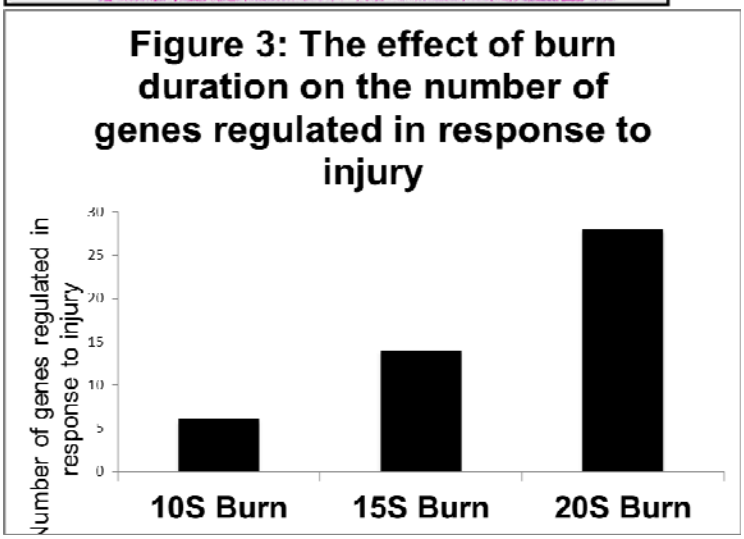
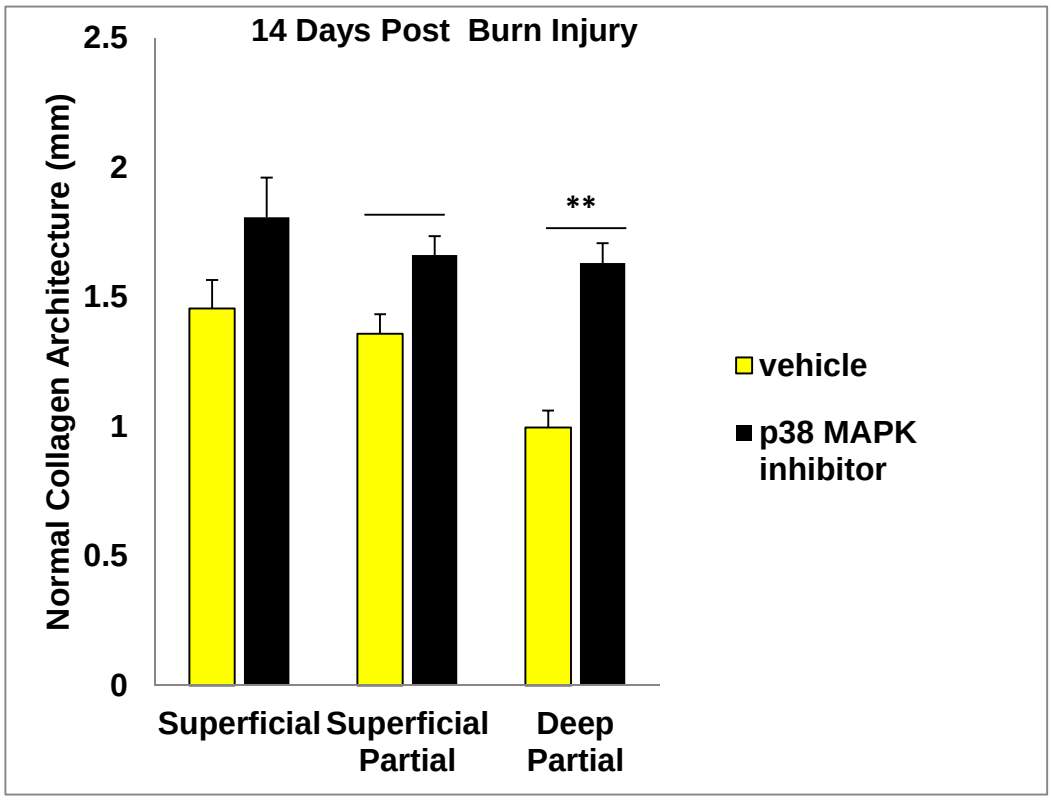


Table 2 Gene Symbol	Gene Name	Fold Regulation	p-value
CCL2	Chemokine (C-C motif) ligand 2	2.9163	0.03716
CCN2	Connective tissue growth factor	3.1712	0.029101
CTNNB1	Catenin (cadherin-associated protein), beta 1	4.1371	0.013599
CTSK	Cathepsin K	2.764	0.000102
CXCL2	Chemokine (C-X-C motif) ligand 2	2.3234	0.023357
EDN1	Endothelin 1	2.2318	0.002653
EGF	Epidermal growth factor	2.3432	0.00295
HBEGF	Heparin-binding EGF-like growth factor	6.0271	0.000001
IL10	Interleukin 10	4.9112	0.048101
ITGA5	Integrin, alpha 5	2.4303	0.010235
ITGAV	Integrin, alpha V	3.0288	0.000075
ITGB1	Integrin, beta 1	2.1722	0.001741
ITGB6	Integrin, beta 6	6.3906	0.002543
PLAUR	Urokinase plasminogen activator surface receptor-like	6.5236	0
LOC100627044	WNT1-inducible-signaling pathway protein 1-like	4.5379	0.02312
WNT5A	Wingless-type MMTV integration site family, member 5A	4.6713	0.010261
MMP2	Matrix metallopeptidase 2	2.75	0.000935
MMP7	Matrix metallopeptidase 7	6.4089	0.003184
MMP9	Matrix metallopeptidase 9	41.2811	0.010249
PTGS2	Prostaglandin-endoperoxide synthase 2 (COX2)	8.3067	0.002638
PLAT	Plasminogen activator, tissue	2.2742	0.018634
PLAU	Plasminogen activator, urokinase	2.482	0.007998
SERPINE1	Serpin peptidase inhibitor, clade E	4.9092	0.000183
TGFB1	Transforming growth factor, beta 1	2.5048	0.003944
TGFB3	Transforming growth factor, beta 3	2.5445	0.000146
TNC	Tenascin C	5.2347	0.012953
ACTB	Actin, beta	2.2379	0.000903
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	2.131	0.00359

Another goal of this grant was to determine the effects of topical p38MAPK inhibition on wound repair after burn injury in the female red Duroc pig. Wounds were treated with either topical p38MAPK inhibitor or vehicle twice daily. Outcomes measured included wound closure and burn depth. Wound closure was determined by measuring wound area with percent wound closure equal to [(Area of original wound – Area of actual wound)/Area or original wound x 100]. Burn depth was determined by measuring the thickness of the residual dermis layer on hematoxylin and eosin stained wound sections from four consecutive non-overlapping fields of the wound bed. Topical p38MAPK inhibition significantly accelerated wound closure ($p<0.001$) on day 3 for the superficial and superficial partial-thickness burns and on day 14 for the deep

partial-thickness burns. Moreover, p38MAPK inhibition also significantly decreased burn depth on day 14 for the superficial partial-thickness burns ($p<0.007$) and the deep partial-thickness burns ($p<0.0009$) (Fig 4).

Figure 4: Topical p38 Inhibition Decreases Burn Depth



In summary, we have complete goal #1, establishing the female red Duroc pig model burn. Goals 2, 3, and 4 (please refer to "What were the major goals of the project?" section) are in progress.
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What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

- Ann Hocking PhD, learned the pig model and burn techniques.
- Adelaide Warsen learned biostatistical analyses and team work
- Toni Griffin, a T32 fellow worked in our lab and learned wound healing principals.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."
The attached abstract was presented at the annual meeting of The Wound Healing Society

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

- Continue to progress with goals 2, 3, and 4.
- The plan is to expand the gene expression studies of Pg004, Pg005, and Pg008 (table 1). We will compare the inflammatory and wound gene expression between the treatment (p38 MAPK inhibitor group) and control groups in the 10, 15, and 20 second burns.
- For Pg006 and Pg007, we will compare the inflammatory signaling in various burn depths with or without treatment (topical p38 MAPK inhibitor). We will also examine hair-follicle apoptosis.
- We will complete our wound closure data for Pg004, Pg005, and Pg008. We will also examine the H&E slides and complete the data for depth of injury and thickness of intact collagen. We will also examine the thickness of the inflammatory area.
- In April-May 2016 we will perform another animal experiment with 8 pigs.
- We will complete two manuscripts, one on establishing the red Duroc burn model and on the effect of p38 MAPK inhibition.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."
The idea of using the red Duroc porcine model to study the relationship between inflammation and scarring adds to the novelty of the current grant. Using an animal model for wound healing that mimics the human disease is an exciting approach in elucidating the molecular mechanisms of normal and abnormal wound healing (hypertrophic scar) pathophysiology and identifying new therapeutic agents. Establishing the red Duroc pig model burn model as the appropriate wound healing model that resembles human response will assist other investigators and companies that need a model to test their product. The ability to have a standard animal model for burn wound healing may bring an exciting new era in the investigation and elucidating the molecular mechanisms of hypertrophic scar pathophysiology and developing therapeutic agents.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."
Please see above

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

No technology transfer

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."
Pending. The ultimate goal of this grant is to develop a topical therapy that reduces end-organ dysfunction, improves wound healing, and attenuates scar formation in patients with burn injuries.

5. CHANGES/PROBLEMS:
If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change
The approach is the same

Actual or anticipated problems or delays and actions or plans to resolve them
There have been no significant complications with our pig model. We did not have any wound infection or death in the pig model last year. The anesthesia and pain management is going well.

Changes that had a significant impact on expenditures
None

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
The current number of pigs for AIM 1 and 2 are now 44 pigs. Aim 3 pig numbers are 8 pigs; therefore, the total pig number been reduced to 52 for the entire 4 years (already approved).

Significant changes in use or care of human subjects

No human subjects

Significant changes in use or care of vertebrate animals.

None

Significant changes in use of biohazards and/or select agents

None

6. PRODUCTS: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."
Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Saman Arbabi, MD, MPH
Project Role: PI
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 1
Contribution to Project: He will provide the overall supervision and direct the animal studies.
Change in effort: From 10% to 8% as of June 1, 2015

Name: Anne Hocking, PhD
Project Role: Co-Investigator
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 2
Contribution to Project: She will provide the overall supervision of the cellular and molecular studies proposed in the current research plan.
Change in effort: From 10% to 20% as of June 1, 2015

Name: Adelaide Warsen, MS
Project Role: Research Scientist
No Change in effort

Name: Noah Ogbi, MS
Project Role: Lab Technician
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 6
Contribution to Project: Research Assistance

Change in effort: Added at 50% as of June 1, 2015

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Nothing to report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable;